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Positive Association Between a DNA Sequence Variant in the Serotonin 2A Receptor Gene and Schizophrenia

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Sixty-two patients with schizophrenia and 96 normal controls were investigated for genetic association with restriction fragment length polymorphisms (RFLPs) in the serotonin receptor genes. A positive association between the serotonin *2A* receptor gene (*HTR2A*) and schizophrenia was found, but not between schizophrenia and the serotonin *1A* receptor gene. The positive association we report here would suggest that the DNA region with susceptibility to schizophrenia lies in the *HTR2A* on the long arm of chromosome 13.

KEY WORDS: association study, schizophrenia, serotonin *2A* receptor, serotonin *1A* receptor, chromosome 13

INTRODUCTION

Atypical neuroleptics (e.g., clozapine and risperidone), which have strong effects on both positive and negative symptoms of schizophrenia, have a high potency to block the serotonin *2A* receptor [Meltzer et al., 1989; Leysen et al., 1992]. The effect of selective serotonin *2A* receptor antagonists (e.g., ritanserin) on negative symptoms of schizophrenia suggests that the serotonin *2A* receptor might be involved in the pathophysiology of schizophrenia [Gelders et al., 1986]. Mita et al. [1986] found a significant decrease in serotonin *2A* receptor density in the postmortem brain of chronic schizophrenics who had not been treated with neuroleptics. Recently a cDNA which encodes the serotonin *2A* receptor has been isolated and localized to chromosome 13 q14-q21 [Hsieh et al., 1990; Saltzman et al., 1991; Chen et al., 1992]. Hallmayer et al. [1992] investigated a large Swedish family with 31 affected members to test a linkage of schizophrenia with the serotonin *2A* receptor gene (*HTR2A*) and other DNA markers on chromosome 13, providing strong evidence against a linkage between schizophrenia and DNA markers on chromosome 13, including the *HTR2A*.

The serotonin *1A* receptor is known to be responsible for symptoms of anxiety and depressive mood. Nemonapride, which has effects on negative symptoms of schizophrenia, was found to have a higher affinity with the serotonin *1A* receptor than buspirone [Fujiwara et al., 1992]. A cDNA encoding the serotonin *1A* receptor has been isolated and localized to chromosome 5 q11.2-13 [Kobilka et al., 1987]. The linkage analysis in 5 families with affective disorder excludes involvement of the serotonin *1A* receptor gene (*HTR1A*) with affective disorder [Curtis et al., 1993]. However there has been no association or linkage study dealing with schizophrenia at the *HTR1A*.

The genes for serotonin receptors can be regarded as candidate genes susceptible for schizophrenia. Therefore we studied the association between schizophrenia and serotonin receptor genes.

MATERIALS AND METHODS

We studied DNA markers for the serotonin *2A* and *1A* receptors in 62 (36 males and 26 females) biologically unrelated patients with schizophrenia. The diagnosis of schizophrenia was made according to DSM-III-R by 2 psychiatrists independently without knowing the results of DNA typing. Thirty-nine patients had a family history of schizophrenia. Ninety-six (38 males and 58 females) controls were recruited from our medical and laboratory staff. None of the controls had a personal or a family history of psychiatric disorders. All patients and the controls were ethnically Japanese. The mean age was 45.0 ± 11.8 (±SD) years for the patients and 37.1 ± 14.4 years for the controls. The mean age of onset was 26.5 ± 7.5 years. All gave informed consent prior to the study.

Genomic DNA was extracted from a 20 ml heparinized venous blood sample using the phenol chloroform method. The *HTR2A* showed an MspI polymorphic site at position 102. A genomic DNA fragment

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corresponding to nucleotide −24 to 318 of the *HTR2A* was amplified by polymerase chain reaction (PCR). PCR was carried out with the primers 5′-TCTGCTACAAGTTCTGGCTT-3′ and 5′-CTGCAGCTTTTTCTCTAGGG-3′. The PCR reaction was carried out using 1 μg genomic DNA, 2.2 μM of each primer, and 1.5 mM $MgCl_2$ in a final volume of 50 μl. The sample was subjected to 3 cycles of 3 minutes at 94°C, 45 seconds at 60°C, and 1.5 minutes at 72°C, then 35 cycles of 1 minute at 94°C, 45 seconds at 60°C, and 1.5 minutes at 72°C [Warren et al., 1993]. The PCR products were digested by MspI. Electrophoreses of the digests were carried out with 3% agarose gel and stained with ethidium bromide.

The *HTR1A* showed an RsaI polymorphic site at position 294. A genomic DNA fragment corresponding to nucleotide 192 to 557 of the *HTR1A* was amplified by PCR. PCR was carried out with the primers 5′-CGCTCCCTGCAGAACGTGGC-3′ and 5′-CATGCGTCGGGGTCCGAGCGGTCTTC-3′ [Warren et al., 1992]. The PCR reaction was carried out using the same method as the *HTR2A* amplification. The PCR products were digested by RsaI. Electrophoreses of the digests were carried out with 3% agarose gel and stained with ethidium bromide. The significance for genetic association of the serotonin receptor polymorphism was estimated by the chi-square test. The relative risk of the marker was estimated by Woolf's [1955] method. *P* values less than 0.05 were considered to be significant.

RESULTS

Digestion of the 342 bp PCR product of *HTR2A* with MspI yielded a 342 bp for allele A1 and 126 and 216 bp products for A2. We found that the allele frequencies and the frequencies of genotypes were not significantly different from those expected from the Hardy-Weinberg equilibrium. The frequency of genotype A2A2 was higher in the patients than in the controls ($P < 0.05$). The frequency of the A2 allele was higher in the patients than the controls ($P < 0.05$). The relative risk of A2A2 homozygotes for schizophrenia was 2.86 ($P < 0.01$; Table I).

Digestion of the 366 bp PCR product of *HTR1A* with RsaI yielded 211 and 155 bp products for allele A1 and 103, 108, and 155 bp products for allele A2. We found that the allele frequencies and the frequencies of genotypes were not significantly different from those expected from the Hardy-Weinberg equilibrium. We found no significant difference in the number of each genotype and allele frequencies between the patients and the controls (Table II).

TABLE I. *HTR2A* Genotypes and Allele Frequencies in Patients With Schizophrenia and the Controls†

	Genotypes			Allele frequencies	
	A1/A1	*A1/A2*	*A2/A2*	*A1*	*A2*
Patients (n = 62)	18	26	18*	0.500	0.500*
Controls (n = 96)	34	50	12	0.615	0.385

†*A1* allele corresponds to absence of MspI restriction site and *A2* to its presence.
*$P < 0.05$.

TABLE II. *HTR1A* Genotypes and Allele Frequencies in Patients With Schizophrenia and the Controls*

	Genotypes			Allele frequencies	
	A1/A1	*A1/A2*	*A2/A2*	*A1*	*A2*
Patients (n = 62)	60	2	0	0.984	0.016
Controls (n = 96)	88	7	1	0.953	0.047

**A1* allele corresponds to absence of RsaI restriction site and *A2* to its presence.

DISCUSSION

To look for a possible association between schizophrenia and the serotonin receptors, we examined a series of biologically unrelated schizophrenic patients and controls using restriction fragment length polymorphisms (RFLPs) at the *HTR2A* and the *HTR1A*. A positive association between *HTR2A* and schizophrenia was found, but not between *HTR1A* and schizophrenia.

These findings would suggest the presence of a coding mutation in linkage disequilibrium with the tested sequence variant. The coding mutation might predispose to the development of schizophrenia through effect on the receptor function. However, we are aware that these results might be due to chance and that replication of the findings is crucial.

The discrepancy between the negative linkage data presented by Hallmayer et al. [1992] and the positive finding in the present study can be explained by a gene effect which is significant but small, and therefore, escapes detection in a conventional linkage study.

The association we report here would suggest that the DNA region with susceptibility to schizophrenia lies in the MspI site within the *HTR2A* at the long arm of chromosome 13.

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